

IDENTIFICATION OF A EUCALYPT FRAGMENT, BASED ON ANATOMY OF LEAF AND STEM

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ABSTRACT: A leafy fragment of a eucalypt from Fenelon Island was treated as a test case for the use of phytoglyphic analysis in identification. Other vegetative characters proved useful in reducing the number of species to be considered (presence or absence of oil glands in the pith) or to confirm the subsequent identification (features of the venation). The fragment is identified as belonging to *E. socialis* or to a species so closely related as to be indistinguishable on vegetative characters alone. Only an examination of reproductive material could substantiate that it is not of *E. socialis* which has, in any case, a wide and as yet undescribed degree of variation.

INTRODUCTION

In 1971 and 1972, Dr. N. M. Wace (Department of Biogeography and Geomorphology, Australian National University) visited Fenelon Island in the Nuyts Archipelago, South Australia (Fig. 1) and collected material of a species of *Eucalyptus*. On neither occasion were fruits or flowers obtainable. The only material provided to us for identification was a leafy twig. The identity of such specimens is of importance to plant geographers but, in general, methods are not currently available for the identification of leafy fragments of eucalypts. We felt, however, that methods we had already developed for examination of the microanatomy of leaf epidermis might help with the problem of the Wace specimens. We have coined the term 'phytoglyph' for the assemblage of microanatomical characters of the surface layers of leaves (Carr, Milkovits & Carr 1971). Experience with a wide range of eucalypts has convinced us of the usefulness of the phytoglyph in the definition of groups or even as diagnostic of the species (Carr, Milkovits & Carr 1971, Carr 1972, unpublished work).

MATERIALS AND METHODS

The specimens listed below were examined critically. Other AD and PERTH specimens, not listed below, were also examined but less critically. Cuticular preparations representative of a very high percentage of all known eucalypts have been prepared and of these a large number have been

examined by scanning electron microscopy as well as by transmitted light microscopy. Other anatomical features have already been examined in specimens of nearly all known eucalypts (e.g. Carr & Carr 1969).

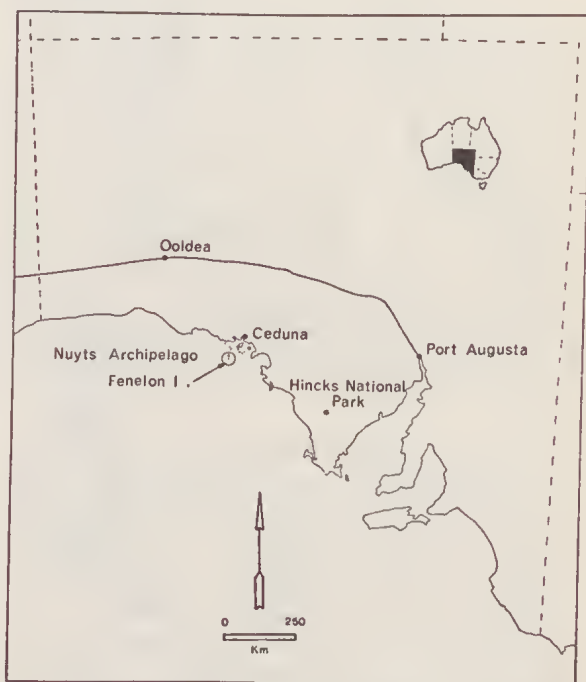


FIG. 1—Map showing South Australian localities mentioned in the text.

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N. M. Wace 286 Fenelon Island, Nuyts Archipelago, S.A., 5.x.1972. Low-growing mallee (45 cm), shoots arising from old burnt stump. From plateau area of island. Soil: sand over limestone (AD).

Eucalyptus socialis F. Muell. ex. Miq. J. B. Cleland s.n. Ooldea, S.A., August 1922 (AD). D. E. Symon 6323, 10.x.1968. Hinck's National Park (AD). D. J. and S. G. M. Carr 747, 33 km E. of Alice Springs, N.T., 27.v.1969.

C. youngiana F. Muell. B. Copley 2668, Ooldea, S.A., 29.vii.1969 (AD).

E. oldfieldii F. Muell. P. G. Wilson 4060, 114 km N. of Kalgoorlie, W.A., 9.iii.1969 (PERTH).

The leafy twigs (Fig. 2) provided by Dr. Wace were already dried. For examination, samples of stems and leaves were boiled in weak Teepol (detergent) until pliable. Hand sections of stems were examined in lactophenol. For cuticular preparations, pieces cut about 1 cm wide from the middle third of a leaf were macerated in hydrogen peroxide. The cuticle was then carefully lifted off and cleaned. For light microscopy, whole cuticle pieces were stained (Sudan Black or Acid Fuchsin) and mounted in glycerine jelly.



FIG. 2—Wace 286, Fenelon Island, $\times 0.5$. The leaf from which a fragment was taken for this study is indicated with an asterisk. It was originally attached to the twig.

For scanning electron microscopy (SEM), pieces of cuticle were mounted on aluminium stubs, shadowed with gold-palladium and viewed in a JSM U3 scanning electron microscope and photographed on Polaroid film. For thin sections, narrow (1-2 mm) slivers of the middle third of a softened leaf were fixed in acrolein (10% solution in water), dehydrated in the series methyl cellosolve, ethanol, propanol, n-butanol and embedded in glycol methacrylate by the method of Feder and O'Brien (1968). Fixation was not essential, since the material had been dried, but even in dried material it seems to stabilize some tissue components. Of course, the cytoplasm is not preserved in dried materials, but fortunately we are concerned here only with the more stable materials of cell walls and cuticles. Thin (1-2 μm) sections were cut with glass knives on a Cambridge ultramicrotome, stained with the periodic acid-Schiff reagent and by toluidine blue (Feder & O'Brien, loc. cit.) and mounted in Depex. Photography of stained cuticles and leaf sections was on Agfa-Gaevert film 49 C 65 using Nikon optics. For venation studies, leaves were cleared in dimethyl sulphoxide (DMSO) and photographed with a Zeiss Tessovar. As a clearing agent, DMSO has the advantage of being non-destructive; it can readily be removed from the leaf which can then be dried and returned to the herbarium sheet (Fig. 2). Its only disadvantage is that it is dangerous to health.

RESULTS

Most herbarium specimens of eucalypts are essentially incomplete, and the tacit assumption is made that identification of an unknown is complete when a number of its characters tally with those of a known species. Only if a complete suite of material were available and if the characters and range of variation of each described species were completely known could the possibility be ruled out that an unknown specimen might be of an undescribed species. In the case of the vegetative specimen collected by Wace we must not rule out the possibility, however unlikely, that it is of an undescribed species. With this proviso we can move to the alternative hypothesis, that it is of a species already described, and try to equate it with a known species. The most likely species to be investigated are those which occur nearby on the mainland.

Wace 286 consists of leaves and a short length of stem. The leaves are isolateral, glaucous and broadly-ovate. The stems have intranodes. The specimen is too incomplete to show whether the

shoot of which it forms part is of adult form or of an intermediate stage of development. However, taking into account the reported size of the plant from which it was taken, there is a possibility that the material is of intermediate form.

An examination of the stems reveals that the pith does not contain oil glands. We have shown (Carr & Carr 1969) that the presence or absence of pith glands is a valuable character for the identification of eucalypts. In the present instance, the absence of oil glands from the pith enables us to exclude about half of the South Australian species listed by Boomsma (1972) from consideration. Even a cursory examination of the leaf venation is sufficient to exclude South Australian members of the Renantherae. All of these have characteristically looped marginal veins distant from the margins and costal veins which intersect the midrib at a very low angle.

A preliminary examination of the leaf epidermis by light microscopy reduces the number of species to be considered to the members of a small group all of which are included in Bisectae (Carr & Carr 1969). The chief epidermal characters which distinguish this group from the other species of Bisectae which lack pith glands are the prominently sculptured cuticle and aggregation of stomata into groups or short lines. Other epidermal characters can be invoked to support the distinction but it is not necessary to discuss them at this point.

From this initial examination it was decided that Wace 286 resembled two species (*E. youngiana* and *E. socialis*) which occur on the adjacent mainland in South Australia and another (*E. oldfieldii*) which occurs only in Western Australia. The phytoglyphic characters which the four species have in common are as follows. The guard cells are rather deeply set and are not visible in SEM preparations viewed from the outside of the cuticle because they are over-arched by broad outer stomatal ledges (Pl. 10, 11, figs. 10-13). The ledges themselves are inserted below the general level of the surface of the cuticle. They are smooth and the orifice they surround is narrow. The depressions in which the outer stomatal ledges are inserted are more or less circular to elliptical.

Each stoma is subtended by five or six, sometimes seven, subsidiary cells. The outer walls of the subsidiary cells are strongly thickened, the thickenings projecting above the surface as mounds. The ordinary epidermal cells have similar, but less prominent, thickenings. The thickenings stain deeply with the dyes used (Pl. 10, figs. 3-10). In the light microscope those of the ordinary epidermal cells are seen to be

circular in outline but those of the subsidiary cells vary between circular and somewhat angular. The thickenings of the subsidiary cells appear to form rosettes around each stoma.

When the differences between the species are considered it is found that *E. oldfieldii* stands apart from the others because the cuticular thickenings of the ordinary epidermal cells contain deeply-staining lenses. Further, in this species, the thickenings of the subsidiary cells tend to be separate. In the Wace specimen, *E. socialis* and *E. youngiana* they are more or less confluent.

The stomata of *E. youngiana* are larger than those of the other species but stomatal size, in our experience, is not an invariant character of eucalypt species. However, *E. youngiana* has less prominent cuticular thickenings than the other species. SEM shows evident similarity between the Wace specimen and *E. socialis* in the angularity and disposition of the cuticular thickenings around the stomata (Pl. 11, figs. 11-14). On the basis of light microscopy of epidermal preparations and of SEM of surface features we may confidently exclude *E. oldfieldii* from consideration and tentatively identify the Wace specimen with *E. socialis*. Median sections through stomata add evidence which strongly supports this suggestion (Pl. 12, figs. 15-20).

The median profile of the stomatal space is, as we will show elsewhere, characteristic of the species. The large blunt, vertically orientated outer stomatal ledges of *E. youngiana* are quite unlike those of the other species (Pl. 12, figs. 15-18). The recurved outer stomatal ledges of *E. oldfieldii* and their position in relation to the guard cells distinguish this species from the others. It is also to be noted that the stomata in *E. oldfieldii* are smaller, and those of *E. youngiana* larger, than those of *E. socialis* and of Wace 286. It is clear from comparison of Pl. 12, figs. 15 & 16 and of Pl. 12, figs. 19 & 20 that the closest match for the Wace specimen is with *E. socialis*.

Finally, an examination of the venation of the leaves (Pl. 13, figs. 21-28) supports the identification. The low vein angles of the Wace specimen (circa 40°) are approached only by those of *E. socialis* (circa 43°). This may not be an important point since the comparison is made between only a single specimen and a few others and we have no means of telling whether any of them represent means or extremes of the range of vein angles characteristic of the species or even of a single tree. More reliable is the pattern of venation. The general venation patterns of the three species are similar. However, there are minor features of the veins close to the margin in

E. socialis and the Wace specimen which are not as those in the other species. In the Wace specimen and in *E. socialis* the minor veins which occur between the marginal vein and the edge of the leaf are reticulate and the oil glands of this part of the leaf are in more than one row. In *E. youngiana* and *E. oldfieldii* the minor veins in this part of the leaf are either simple and run from the marginal vein to the edge of the leaf or form single closed loops. The oil glands usually occur in only one row.

DISCUSSION AND CONCLUSION

With the proviso made above (Results) we believe that the specimen collected from Fenelon Island by Wace can confidently be identified as *E. socialis*. Some degree of variation in the phytoglyphic characters (as in others, e.g. venation) must be expected as a measure of the general range of variation within the species. An examination of other specimens of *E. socialis* confirms, for instance, that there is variation in that species in the degree of angularity of the thickenings of the cuticles. The characters of the specimens of *E. youngiana* and *E. oldfieldii* used to illustrate this study are, however, generally consistent with those of other specimens of these species which we have examined. Observed similarities to and differences from the Wace specimen can be confirmed. Geographical isolation of the Wace eucalypt on Fenelon Island could lead to incipient or full speciation but we have no reason from our examination of the material provided to suppose that speciation has progressed to any marked degree. In any case, to establish it would require critical biological as well as anatomical examination of more than a few specimens of the species in question.

We have shown that the content of taxonomic information in eucalypt leaves is very much larger than is generally supposed (Carr, Milkovits & Carr 1971). The methods we have applied are evidently sufficient in at least some cases, to enable identification of eucalypts to be made to the level of the species. Moreover, there are general phytoglyphic resemblances between the members of groups of species which on other grounds may be regarded as natural. In the SEM views (Pl. 11, figs. 11-14) we recognize certain

similarities of pattern which would lead us to group the species used in this study and which are different from the patterns found in other groups. Similarly, in the sections (Pl. 12, figs. 15-20) there are microanatomical characters which may be unique to this group. We have not seen, for instance, the extraordinary intrastomatal cuticular thickenings (Pl. 12, figs. 19-20) forming the line of closure of the stoma in any other group of eucalypts so far examined. Thus, even when identification to the species cannot be certainly made, a specimen examined by our methods may with certainty be referred to a group of species.

In many cases, and with experience, sufficient of the phytoglyphic characters can be seen in fairly crude, temporary preparations with the light microscope to enable rapid decisions to be made concerning unknown or dubious specimens. Critically, and for convincing demonstration, the methods described above may be applied but, carried out as a routine, they are not unduly time-consuming. The fact that an examination can be carried out using a fragment of a single leaf (Fig. 2) is advantageous since it is almost non-destructive to the whole specimen. It is to be hoped that more general use will be made of the abundant and reliable information contained in the microanatomy of the leaf, particularly in the phytoglyph, to solve difficult problems in the eucalypts as well as in other groups of plants.

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DESCRIPTION OF PLATES 10-13

PLATE 10

FIGS. 3-10—Stained cuticles examined by light microscopy. Wace (W) = Wace 286. *E. socialis* = Cleland, Ooldea. The arrow, Fig. 6, indicates the lens-like thickenings of the cuticle over ordinary epidermal cells of *E. oldfieldii*. Figs. 3-6, $\times 90$. Figs. 7-10, $\times 280$.

PLATE 11

FIGS. 11-14—Scanning electron micrographs of cuticles. E.S. = *E. socialis* (Symon 6323). The other abbreviations as in Figs. 7-10. All at $\times 350$.

PLATE 12

FIGS. 15-20—Median sections through stomata. E.S. = *E. socialis* (Cleland, Ooldea); osl = outer stomatal ledge. The other abbreviations as in Figs. 7-10. The arrows in Figs. 17, 19 and 20 indicate the cuticular projections which form the line of closure of the stomatal pore. Figs. 15-18, $\times 200$. Figs. 19, 20, $\times 350$.

PLATE 13

FIGS. 21-28—Venation of leaves. E.S. = *E. socialis* (Cleland, Ooldea); the other abbreviations as in Figs. 7-10. Arrows in Figs. 25-28 indicate the minor venation between the marginal vein and the margin of the leaf. Figs. 21-24, $\times 3$. Figs. 25-28, $\times 6$.

